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Comparative analysis of male androgen responsiveness to social environment in birds: the effects of mating system and paternal incubation

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Abstract

Male androgen responses to social challenges have been predicted to vary with mating system, male–male aggressiveness, and the degree of paternal investment in birds ("challenge hypothesis," Am. Nat. 136 (1990), 829). This study focused on the interspecific predictions of the challenge hypothesis. Comparative methods were used to control for effects of the phylogenetic relatedness among the sampled species. Male androgen data of 84 bird species were collected from literature records on seasonal androgen patterns. From these, the androgen responsiveness (AR) was calculated as described in the original challenge hypothesis (i.e., maximum physiological level/breeding baseline). Scatterplots of AR versus mating strategy, male–male aggressiveness, and the degree of paternal care confirmed the expected interspecific patterns. When phylogenetic analyses were performed among all of the sampled species, the effects of paternal investment disappeared while the AR remained covarying to a high degree with mating system and male–male aggressiveness. Although these mechanisms may be different at the intraspecific level, this suggests that interspecific differences of AR in male birds may have evolved in response to changes of mating strategies, rather than in response to altered paternal duties. However, control for phylogeny among the subsample of 32 passerine species revealed that if any paternal investment contributed to the observed variance in AR, then the change from "no male incubation" to "male shares incubation duties" represented the most effective, whereas the male's contribution to feeding offspring did not explain the observed variation of AR.
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To optimize their reproductive output, males have to trade-off between mating, which requires high levels of androgens, and parenting, which requires low androgen levels (Hegner and Wingfield, 1987). Variation of androgen levels above a reproductive baseline (i.e., the minimum amount needed for the expression of secondary sex characters, the maturation of the gonads, and the expression of reproductive behavior) within wild populations of birds may be explained by the flexibility of the individual androgen responsiveness (AR) to the social environment (Wingfield et al., 1990). In particular, it was proposed that males'

androgen levels should be responsive to social challenges imposed by other males in competition for mates and territories ("challenge hypothesis"; Wingfield et al., 1990). In monogamous species with paternal care, male–male competition for mates should be lower than in polygynous species without paternal care. Therefore, one of the predictions of the challenge hypothesis is that the AR to social challenges should vary with mating strategy and the degree of paternal investment (Wingfield et al., 1990, 2000). Monogamous male birds with a high degree of paternal care are predicted to have low breeding baseline androgen levels and will remain highly responsive to social challenges (e.g., territorial intrusions) throughout the breeding season. In contrast, males with a high degree of polygyny and low or

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no paternal care are predicted to have high androgen levels throughout all breeding phases and thus, to exhibit only small further increases in response to social challenges (Beletsky et al., 1989). These predictions have been confirmed by a number of correlational studies in free-living bird populations (Rissman and Wingfield, 1984; Wingfield, 1984a; Wada et al., 1999; Wikelski et al., 1999; Hirschenhauser et al., 2000; Wingfield et al., 2000). Moreover, exogenous administration of testosterone was shown to inhibit the transition from mating to paternal behavior, and to promote polygynous mating strategies in monogamous biparental populations of various passerine species (Silverin, 1980; Wingfield, 1984b; Hegner and Wingfield, 1987; Dittami et al., 1991; Ketterson and Nolan, 1992; De Ridder et al., 2000). However, a hormonal signal may not only interact with behavior, physiology, and morphology, but in addition, it may involve potential "costs" in terms of overall fitness, such as direct and indirect energetic costs, immunosuppression, increased mortality, and reproduction failure (Marler and Moore, 1988; Dufty, 1989; Wingfield, 1990; Ros et al., 1997; Wingfield et al., 1999a; Peters, 2000). As these changes in fitness occur where the same hormone mediates antagonistic traits, androgens can be viewed as physiological mediators of the trade-off between investment in male–male competition or in paternal care (Hegner and Wingfield, 1987; Ketterson and Nolan, 1992). Therefore, hormonal mechanisms, like other phenotypic traits, may be targets of selection (Ketterson and Nolan, 1992; Houck and Woodley, 1995). At this stage it is important to point out the importance of distinguishing between the intraspecific and the interspecific level of the predicted interactions because correlations among traits within a species may not necessarily correspond to correlations among species (Ptacek and Travis, 1998). For instance, among the cooperatively breeding *Acrocephalus* warblers helping species occur in poor habitats, while within species helpers are more numerous at good sites (Leisler et al., 2002). Accordingly, the predicted interactions between mating strategy, paternal care, and AR to social challenges at the intraspecific level do not necessarily have to act equally at the interspecific level. Here, the predictions of the challenge hypothesis were tested at the interspecific level. We analyzed the effects of mating strategy and degree of paternal care on AR to social challenges in males from different bird species and added a control for the phylogenetic relatedness among the sampled species.

Methods

We carried out a literature survey collecting measures of the potential AR to social challenges of males from different bird species in order to reproduce and to expand the size of the original sample (Wingfield et al., 1990: 20 species; Wingfield et al., 2000: 60 species). The references given in the original challenge hypothesis were scanned as well as the articles found in online database surveys (Cambridge

Table 1
Distribution of the different categories of mating system and paternal care among the 84 sampled species

Mating system	
Monogamous	72.6%
Polyandrous	3.6%
Polygynous	20.2%
Promiscuous	3.6%
Parenting system	
Nonpaternal	16.7%
Biparental	76.2%
Paternal	7.1%

University Abstracts; IDEAL-Library; NISC-Biblioline; PubMed) that reported territorial intruder experiments (situational) or seasonal androgen patterns. From each paper the following measures were taken: (1) the "nonbreeding baseline" androgen level (level A sensu Wingfield et al., 1990: photorefractory levels or, if not given, the lower limit of reliable measurements of the immunoassays); (2) the "breeding baseline" level (level B sensu Wingfield 1990 et al.: the average androgen level throughout the different breeding phases, including parenting); and (3) the "physiological maximum" (level C sensu Wingfield, 1990 et al.: specific responses to territorial challenges, if available, or the highest androgen level observed during peak mating phases, where high frequencies of territorial male–male interactions may be assumed). Based on these measures, the potential AR for each species was expressed as the relative ratio (C-A)/(B-A) as proposed by Wingfield et al. (1990). For example, a large AR indicates that males in this species respond to territorial intruders with large androgen increases from low breeding baseline levels to the physiological maximum, whereas males from species with smaller AR have already higher breeding baseline androgen levels and, therefore, further increases in response to social stimuli are smaller (Oliveira et al., 2001a). As a rule, when more than one article was available for a given species, the one cited by Wingfield et al. (1990, 2000) was used, and in the case of species not present in Wingfield's database, the most recently published results were selected. When the data needed were not given in the text or in tables, the published figures were scanned and the androgen levels were extrapolated using the UnGraph software package (Biosoft, 1998).

Our database consists of 84 bird species, and is dominated by monogamous and biparental species (61 and 64 species, respectively, Table 1), which reflects the available distribution of mating and parenting systems among birds (Clutton-Brock, 1991) and echoes the distribution of social systems in the sample represented in the challenge hypothesis (Wingfield et al., 2000).

In order to reproduce the patterns from the original challenge hypothesis (Wingfield et al., 1990, 2000), information on male–male aggressiveness (competition for mates and territory), mating strategy, and the degree of

paternal care were collected for each species as given in the original challenge hypothesis (Table 2). The degree of territorial male–male aggressiveness was rated on a three-point scale (low, moderate, high) according to the information given in the scanned article or, where not given there, as classified in Cramp (1998). Regarding mating strategy, species were assigned to one of the four categories: monogamous, polyandrous, polygynous, or promiscuous. Furthermore, the scale of the degree of paternal care (i.e., maternal, biparental, or paternal) was refined, by quantifying the males' specific contribution to the different parental phases, such as incubation and feeding offspring expressed as percentages (Møller and Cuervo, 2000; Table 2). This information was, if not given in the original articles, taken from textbooks (Morse Nice, 1943; Willis, 1972; Glutz von Blotzheim et al., 1981; Welty, 1982; Stacey and Koenig, 1990; Hoyo et al., 1992, 1996; Johnsgard, 1994; Cramp, 1998) or provided by the authors of the original articles. Because of the variables involving offspring feeding, these analyses included data from altricial species only. We chose to analyze the subsample of passerines ($N = 32$ species), as this is a relatively homogenous group with regard to body size, which provided sufficient variability of paternal care systems during the different breeding phases. Numbers of species may vary depending on the information available.

Because AR data were skewed, they were $\log_{10}$-transformed. First, we repeated the analysis performed in the original challenge hypothesis (i.e., Wingfield et al., 1990) by plotting the AR of the 84 species in our database by the "ratio d" (male–male aggressiveness divided by the degree of paternal care). A small ratio d indicates low male–male aggressiveness and a high degree of paternal care in this species (for example *Aptenodytes patagonicus;* Table 2), whereas highest ratios d result from species with highly territorial males and no paternal care (for example *Ptilonorhynchus violaceus;* Table 2). Because we aimed at elaborating the specific contribution of each of the independent variables per se, we subsequently dealt with the mating strategy, the degree of paternal care, and the male–male aggressiveness categories separately.

Control for phylogeny

For the comparative analyses, we employed generalized linear methods as outlined in Dobson (1990). Models of this kind can be extended to include covariances among species due to the evolutionary process (Martins and Hansen, 1997; Rohif, 2001). Thus, the full range and power of this tool is available for statistical analyses in comparative studies, without the problems that pertain to popular algorithmic methods (e.g., independent contrasts), which produce, however, identical results under certain boundary conditions (Rohif, 2001). Multiple regression analysis was used with the independent variables coded as discrete states. In the generalized least squares (GLS) estimations, two error models were considered. The basic assumption for the analyses

was that the phylogenetic variance corresponds to the Brownian motion model (Felsenstein, 1985). However, we also tested a punctuated model. Because the results did not differ between the two models and because it may not be appropriate to estimate the number of speciation events lying between two nodes from phylogenetic trees that cover widely different groups at varying resolution, we only report the results of the gradual model. The phylogeny used was derived from Sibley and Ahlquist (1990), who analyzed DNA–DNA hybridization data. Additional information came from cytochrome b studies by Ellsworth et al. (1996), Patten and Fugate (1998), Helbig and Seibold (1999), Johnson and Lanyon (1999), Kimball et al. (1999), and Klicka et al. (2000).

To control for the effect of phylogenetic relatedness, the independent variables mating system, male–male aggressiveness, and degree of paternal care were entered separately into single and multiple regression analyses (GLS) with AR as the dependent variable (Price, 1997; Garland et al., 1999; Dunn et al., 2001). Mating system was coded with three independent dummy variables (Tabachnik and Fidell, 2001; i.e., polyandrous 1,0,0; monogamous 0,1,0; polygynous 0,0,1; and promiscuous 0,0,0). Male–male aggressiveness was also coded with two dummy variables (i.e., species with low 0,0; moderate 1,0; and high territorial male–male aggressiveness 1,1), and the degree of paternal care was coded with two dummy variables that implied an order of the paternal effort (i.e., species with no paternal care 0,0; biparental species 1,0; and species with full paternal care 1,1). However, to rule out the potential bias due to defining the mating categories as continuous variables with equidistant phenotypes, we also repeated the analyses comparing between two discrete character states of mating system [monogamous ($N = 61$) vs polygynous ($N = 17$)]. The alternative would have been to use ANOVA, equivalent to multiple regression on similar dummy variables, but constrained to sum up to zero (Overall and Spiegel, 1969; Dobson, 1990). Because the data are unbalanced with respect to the factors analyzed, no matter which analysis is used, the problem of colinearity and thus, deciding which variable is more influential remains. Confidence intervals of each category in the final regression models served to determine the significant contributions of each variable to the variance of the response.

Social environment

Because species living at high group densities may better cope with higher frequencies of male–male interactions, the effects of different parameters of the social environment were also included in the analyses. The mode of group living that males of different species were coping with during the breeding season was qualitatively determined [i.e., breeding density: dispersed ($N = 42$), or aggregated nesting ($N = 30$), Westneat and Sherman, 1997; Searcy et al., 1999], as well as group living outside of the breeding

Table 2
List of all sampled species with the measured androgen responsiveness [AR = (C-A)/(B-A)] and the categories classified for the phylogenetic analyses

Species	AR	Mating[a]	Parental care[b]	Territory aggress.[f]	Precocial/ altricial[g]	INC%	FO%	Breeding density	Group living[h]	Pairbond	Free-living/ captive	Seasonal/ situational	Literature[i]
Struthio camelus dom.	2.8	Polygyn	Bipar	High	P	60	—	Dispersed	Mm groups	Seasonal	C	Seasonal	Degen et al., 1994
Dromaius novaehollandiae	2.8	Monog	Patern	Low	P	100	—	Dispersed	Solitary	Seasonal	C	Seasonal	Malecki et al., 1998
Apteryx australis	47.6	Monog	Patern	Low	P	100	—	Dispersed	Solitary	Long-term	F	Seasonal	Cockrem and Potter, 1990*
Colinus virginianus texanus	60.3	Monog	Patern[c]	Low	P	100	—		Mm groups	Seasonal	C	Situational	Vleck and Dobrott, 1993
Tetrao tetrix	2.5	Promisc	Matern	High	P	0	—	Dispersed	Mm groups	None	F	Seasonal	Alatalo et al., 1996
Lagopus lagopus	17.2	Monog	Bipar	High	P	0	—	Dispersed	Solitary	Seasonal	F	Seasonal	Hannon and Wingfield, 1990*
Lagopus mutus hyperboreus	3.7	Polygyn	Bipar	High	P	0	—	Dispersed	Solitary	Seasonal	F	Seasonal	Stokkan and Sharp, 1990*
Meleagris gallopavo	2.7	Polygyn	Matern	High	P	0	—	Aggregated	Solitary	None	C	Seasonal	Lisano and Kennamer, 1977*
Phasianus colchicus versicolor	1.6	Polygyn	Matern	High	P	0	—	Dispersed	Mm groups	None	C	Seasonal	Sakai and Ishii, 1986*
Coturnix c. japonica	1.2	Polygyn	Matern	High	P	0	—		Solitary	Seasonal	C	Seasonal	Ishii et al., 1994
Alectoris rufa	9.7	Monog	Bipar	High	P	30	—	Dispersed	Solitary	Long-term	C	Seasonal	Bottoni et al., 1993
Perdix perdix	14.9	Monog	Bipar	Low	P	0	—	Dispersed	Mm groups	Long-term	F	Seasonal	Fraissinet et al., 1987*
Branta canadensis	3.6	Monog	Bipar	High	P	0	—	Aggregated	Mm groups	Long-term	C	Situational	Akesson and Raveling, 1981*
Anser anser	3.4	Monog	Bipar	High	P	0	—	Dispersed	Mm groups	Long-term	C	Seasonal	Hirschenhauser et al., 1999**
Anser anser dom.	1.5	Polygyn	Bipar	High	P	0	—	Dispersed	Mm groups	Long-term	C	Seasonal	Kotrschal et al., 2000**
Anser indicus	6.0	Monog	Bipar	High	P	0	—	Dispersed	Mm groups	Long-term	F	Seasonal	Dittami, 1981*
Somateria mollissima	3.1	Polygyn	Matern	High	P	0	—	Aggregated	Mm groups	Seasonal	F	Seasonal	Gorman, 1977
Anas platyrhynchus	1.6	Polygyn	Matern	High	P	0	—	Aggregated	Mm groups	Long-term	C	Seasonal	Paulke and Haase, 1978*
Anas platyrhynchus dom.	2.1	Polygyn	Matern	High	P	0	—	Aggregated	Mm groups	Long-term	C	Seasonal	Paulke and Haase, 1978*
Picoides borealis	13.1	Monog	Bipar[d]	Low	A	30	37	Aggregated	Mm groups	Long-term	F	Seasonal	Khan et al., 2001
Ceryle rudis	2.7	Monog	Bipar[d]	High	A	60	60	Aggregated	Mm groups	Seasonal	F	Seasonal	Reyer et al., 1986
Psittacula krameri	2.5	Monog	Bipar	High	A	0	50	Aggregated	Mm groups	Seasonal	F	Seasonal	Krishnapradasart et al., 1988
Streptopelia decaocto	9.7	Monog	Bipar	Mod	A	33	50	Dispersed	Mm groups	Long-term	C	Seasonal	Péczely and Pethes, 1979*
Streptopelia risoria	6.2	Monog	Bipar	Mod	A	50	50	Dispersed		Long-term	C	Situational	O'Connel et al., 1981
Chlamydotis macqueenii	5.1	Monog	Matern	Mod	P	0	—	Dispersed	Mm groups	Seasonal	C	Seasonal	Saint Jalme et al., 1996
Chlamydotis undulata	3.2	Promisc	Matern	Mod	P	0	—	Dispersed	Mm groups	None	C	Seasonal	Saint Jalme et al., 1996
Phalaropus lobatus	32.4	Polyandr	Patern	Low	P	100	—	Dispersed	Solitary	Seasonal	F	Seasonal	Gratto-Trevor et al., 1990*
Phalaropus tricolor	5.9	Polyandr	Patern	Mod	P	100	—	Dispersed	Solitary	Seasonal	F	Seasonal	Fivizzani et al., 1986*
Actitis macularia	5.3	Polyandr	Patern	Mod	P	100	—	Dispersed	Solitary	Seasonal	F	Seasonal	Fivizzani and Oring. 1986*
Calidris pusilla	16.3	Monog	Bipar	High	P	50	—	Dispersed	Mm groups	Seasonal	F	Seasonal	Gratto-Trevor et al., 1990*
Chionis minor	13.5	Monog	Bipar	Mod	A	0	50	Dispersed	Mm groups	Long-term	F	Seasonal	Burger and Millar, 1980*
Larus occidentalis	14.0	Monog	Bipar	Mod	A	42	57	Aggregated	Mm groups	Long-term	F	Seasonal	Wingfield, 1990*
Falco tinnunculus	12.6	Monog	Bipar	High	A	0	90	Dispersed	Solitary	Long-term	F	Seasonal	Meijer and Schwabl, 1989*
Parabuteo unicinctus	22.2	Monog	Bipar[d]	Low	A	30	50	Aggregated	Mm groups	Long-term	F	Seasonal	Mays et al., 1991*
Phaeton rubricauda	5.7	Monog	Bipar	High	A	50	50	Aggregated	Solitary	Long-term	F	Seasonal	Lormée et al., 2000
Sula dactylatra	16.0	Monog	Bipar	High	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Lormée et al., 2000
Sula sula	4.0	Monog	Bipar	High	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Lormée et al., 2000
Sula nebouxii	6.4	Monog	Bipar	High	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Wingfield et al., 1999b
Phalacrocorax capensis	1.8	Monog	Bipar	High	A	50	50	Aggregated	Mm groups	Seasonal	F	Seasonal	Berry et al., 1979*
Eudyptes chrysolophus	6.4	Monog	Bipar	Low	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Williams, 1992*
Megadyptes antipodes	14.1	Monog	Bipar	Low	A	50	50	Aggregated	Solitary	Long-term	F	Seasonal	Cockrem and Seddon, 1994
Pygoscelis adeliae	9.2	Monog	Bipar	Low	A	63	50	Aggregated	Mm groups	Long-term	F	Seasonal	Groscolas et al., 1986*
Pygoscelis papua	21.2	Monog	Bipar	Low	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Williams, 1992*
Aptenodytes forsteri	16.4	Monog	Bipar	Low	A	100	50	Aggregated	Mm groups	Long-term	F	Seasonal	Groscolas et al., 1986*
Aptenodytes patagonicus	24.8	Monog	Bipar	Low	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Mauget et al., 1994*
Diomedea chrysostoma	15.0	Monog	Bipar	Low	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Hector et al., 1986a*
Diomedea exulans	5.4	Monog	Bipar	Low	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Hector et al., 1986b*
Diomedea melanophris	27.8	Monog	Bipar	Low	A	50	50	Aggregated	Mm groups	Long-term	F	Seasonal	Hector et al., 1986a*
Hylophylax naevioides	19.4	Monog	Bipar	High	A[P]	50	50	Dispersed	Solitary	Long-term	F	Seasonal	Wikelski et al., 1999
Ptilonorhynchus violaceus	2.18	Promisc	Matern	High	A[P]	0	0	Dispersed	Solitary	None	F	Situational	Borgia and Wingfield, 1991*
Malurus cyaneus	2.8	Monog	Bipar[e]	High	A[P]	0	50	Aggregated	Mm groups	Seasonal	F	Seasonal	Peters, 2000

(continued on next page)

Table 2 (*continued*)

Species	AR	Mating[a]	Parental care[b]	Territory aggress.[f]	Precocial/ altricial[g]	INC%	FO%	Breeding density	Group living[h]	Pairbond	Free- living/ captive	Seasonal/ situational	Literature[i]
Lanius collurio	8.4	Monog	Bipar	High	A[P]	0	56	Dispersed	Solitary	Seasonal	F	Seasonal	Fornasari et al., 1992*
Pachycephala rufiventris	19.8	Monog	Bipar	High	A[P]	36.2	50				F	Situational	McDonald et al., 2001
Corvus frugilegus	6.1	Monog	Bipar	Mod	A[P]	0	69	Aggregated	Mm groups	Long-term	F	Seasonal	Péczely and Pethes, 1982*
Aphelocoma coerulescens	7.5	Monog	Bipar[d]	Mod	A[P]	0	60	Dispersed	Mm groups	Long-term	F	Seasonal	Schoech et al., 1991*
Aphelocoma californica	9.1	Monog	Bipar	Mod	A[P]	0	50	Dispersed	Mm groups	Long-term	F	Seasonal	Vleck and Brown, 1999
Aphelocoma ultramarina	2.5	Polygyn	Bipar[e]	High	A[P]	0	50	Dispersed	Mm groups	Long-term	F	Seasonal	Vleck and Brown, 1999
Cinclus pallasii	4.9	Monog	Bipar	High	A[P]	0	50	Dispersed	Solitary	Long-term	F	Seasonal	Kofuji et al., 1993**
Turdus merula	4.8	Monog	Bipar	Mod	A[P]	0	51	Dispersed	Solitary	Seasonal	F	Seasonal	Schwabl et al., 1980*
Saxicola torquata	3.8	Polygyn	Bipar	High	A[P]	0	50	Dispersed	Solitary	Seasonal	F	Situational	Gwinner et al., 1994
Ficedula hypoleuca	7.7	Polygyn	Bipar	Mod	A[P]	0	44	Dispersed	Solitary	Seasonal	F	Seasonal	Silverin and Wingfield, 1982*
Lamprotornis chalybaeus	2.2	Monog	Bipar	High	A[P]	?	?				F	Seasonal	Dittami and Gwinner, 1990
Sturnus vulgaris	10.4	Polygyn	Bipar	Mod	A[P]	24	50	Aggregated	Mm groups	Seasonal	F	Seasonal	Ball and Wingfield, 1987*
Toxostoma curvrostre	6.5	Monog	Bipar	High	A[P]	75	50			Long-term	F	Seasonal	Vleck, 1993*
Campylorhynchus brunneicapillus	6.7	Monog	Bipar	High	A[P]	0	50	Dispersed	Solitary	Seasonal	F	Seasonal	Vleck, 1993*
Parus major	7.7	Monog	Bipar	Mod	A[P]	40	50	Dispersed	Mm groups	Seasonal	F	Seasonal	Silverin, 1990
Acrocephalus scirpaceus	2.7	Monog	Bipar	High	A[P]	12	50	Dispersed	Solitary	Seasonal	F	Seasonal	Dittami et al., 1991
Cettia diphone	2.9	Polygyn	Matern	High	A[P]	0	0	Dispersed	Solitary	None	F	Seasonal	Wada et al., 1999
Passer domesticus	4.6	Monog	Bipar	High	A[P]	39	45	Aggregated	Mm groups	Long-term	F	Seasonal	Hegner and Wingfield, 1986*
Passer rufocinctus	2.4	Monog	Bipar	Mod	A[P]	?	?				F	Seasonal	Dittami and Gwinner, 1990
Lonchura striata dom.	19.5	Monog	Bipar	Low	A[P]	50	50		Mm groups		C	Seasonal	Seiler et al., 1992
Plocepasser mahali	4.9	Monog	Bipar[e]	Mod	A[P]	25	50		Mm groups	Long-term	C	Seasonal	Levin and Wingfield, 1992
Ploceus reichenowi	3.3	Monog	Bipar	Mod	A[P]	0	50		Mm groups	Long-term	F	Seasonal	Dittami and Gwinner, 1990
Ploceus philippinus	2.2	Polygyn	Matern	High	A[P]	0	0	Aggregated	Mm groups	Seasonal	F	Seasonal	Narasimhacharya et al., 1980
Carpodacus mexicanus	6.4	Polygyn	Bipar	High	A[P]	0	30	Dispersed	Solitary	Seasonal	C	Situational	Belthoff and Dufty, 1994
Agelaius phoeniceus	1.2	Polygyn	Matern	High	A[P]	0	5	Dispersed	Mm groups	None	F	Seasonal	Beletsky et al., 1989*
Molothrus ater	2.1	Monog	—	High	—[P]	0	0	Aggregated	Mm groups	None	F	Seasonal	Dufty, 1993*
Melospiza melodia	12.4	Monog	Bipar	High	A[P]	0	71	Dispersed	Mm groups	Seasonal	F	Seasonal	Wingfield, 1994
Zonotrichia leucophrys	3.2	Monog	Bipar	Mod	A[P]	0	40	Dispersed	Solitary	Seasonal	F	Seasonal	Moore, 1982
Junco hyemalis	4.8	Monog	Bipar	High	A[P]	0	50		Mm groups	Seasonal	F	Seasonal	Chandler et al., 1997
Amphispiza bilineata	12.4	Monog	Bipar	High	A[P]	?	50		Mm groups		F	Seasonal	Vleck, 1993*
Spizella arborea	21.0	Monog	Bipar	Mod	A[P]	0	50	Dispersed	Mm groups	Seasonal	F	Seasonal	Hunt et al., 1995*
Calcarius lapponicus	6.8	Monog	Bipar	Mod	A[P]	0	50	Dispersed	Mm groups	Seasonal	F	Seasonal	Hunt et al., 1995*
Plectrophenax nivalis	4.9	Monog	Bipar	High	A[P]	?	50	Aggregated	Solitary	Seasonal	F	Seasonal	Romero et al., 1998

Note. INC, male's contribution to incubation; FO, male's contribution to feeding offspring.

[a] Polygn, polygynous (>5% polygyny); Monog, monogamous (<5% polygyny); Promisc, promiscuous.

[b] Bipar, biperental care; Patern, paternal care; Matern, maternal care.

[c] Study on foster-fathering males. Note that bobwhite quails usually share incubation duties.

[d] Only male helpers.

[e] Helpers from either sex.

[f] High, highly territorial breeding males; Mod, moderately.

[g] A[P], (altrical) passerine species.

[h] Mm groups, multimale groups.

*, in Wingfield et al., 2000 sample; **, data derived from fecal equivalents.

season [species with solitary males ($N = 26$) or species with multimale groups ($N = 52$), Møller and Birkhead, 1993]. The effect of the pairbond type on the specific androgen response rates was also qualitatively determined as "no pairbond" ($N = 7$), "seasonal bond" ($N = 32$), or "long-term bond" ($N = 39$) and was coded with dummy variables as the degree of paternal care. Finally, the precociality of the species was taken into account [altrical ($N = 58$) vs pre-cocial species ($N = 25$)]. Numbers of species may vary depending on the information available.

Results

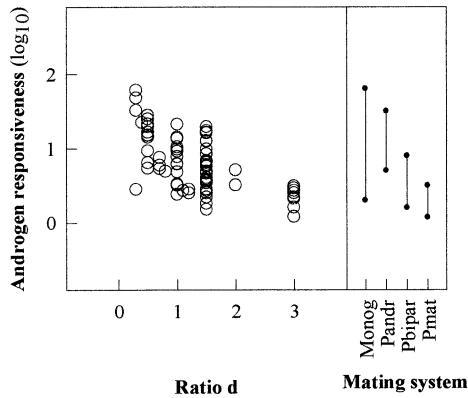Plots of the potential AR from the 84 sampled bird species by the ratio d confirmed the pattern observed by

Fig. 1. Left panel: interspecific pattern of the AR (ratio [C-A]/[B-A]; Wingfield et al., 1990) as determined from the literature among males of 84 bird species plotted by the ratio d (i. e., male–male aggressiveness divided by the degree of paternal care). Right panel: ranges of AR in males from monogamous (Monog), polyandrous (Pandr), polygynous and biparental (Pbipar), and polygynous and maternal (Pmat) species. For references of androgen data sources please refer to Table 2.

Wingfield et al. (1990, 2000) with an increased sample size. That is, males from species with a high degree of paternal care seemed to respond to social challenges with larger androgen increases than species with high male–male aggressiveness and a low degree of paternal investment (Fig. 1). In order to test the effects of each of the parameters involved into the ratio d, the independent variables male–male aggressiveness, degree of paternal care, and mating system were entered into a regression analysis of all observed AR rates. The multiple effect of all variables together satisfied the predicted variation of androgen rates within confidence intervals of ± 4% ($F_{7.76} = 25.96$; $P < 0.0001$). So far, the results could be interpreted as male–male aggressiveness and paternal care contributing the strongest effects on the observed interspecific variation of AR, with polygyny providing only a minor additional explanation.

Control for phylogeny

To control for the effect of phylogenetic relatedness, the independent variables male–male aggressiveness, mating strategy, and degree of paternal care were entered into single and multiple regression analyses (GLS) by comparing the results from using raw data with those from using phylogenetic contrasts. The single effects of mating system and male–male aggressiveness on AR remained significant after adding the phylogenetic information (Fig. 2a and b). Additionally, to rule out potential bias due to the assumption of equidistances between the different phenotypes of a continuous scale for the different mating systems, we compared the AR of males from monogamous species (mean AR ± SEM: 11.18 ± 1.34) with those from polygynous species (mean AR ± SEM: 3.37 ± 0.57; discrete variables that imply no order of the independent variables). Also by using this approach, the effect of mating system on AR

remained robust to incorporating information on the phylogenetic relationship among the species included (without phylogeny: $F_{1,76} = 24.56$; $P < 0.0001$; with phylogeny: $F_{1,76} = 22.28$; $P < 0.0001$). The effect of the degree of paternal care on AR also remained significant (Fig. 2c). However, both analyses (without and with phylogeny) showed that the major variation of AR was explained by the change from no paternal care to at least some paternal care (biparental), whereas the effect of the change from biparental to full paternal care did not remain significant (Fig. 2c).
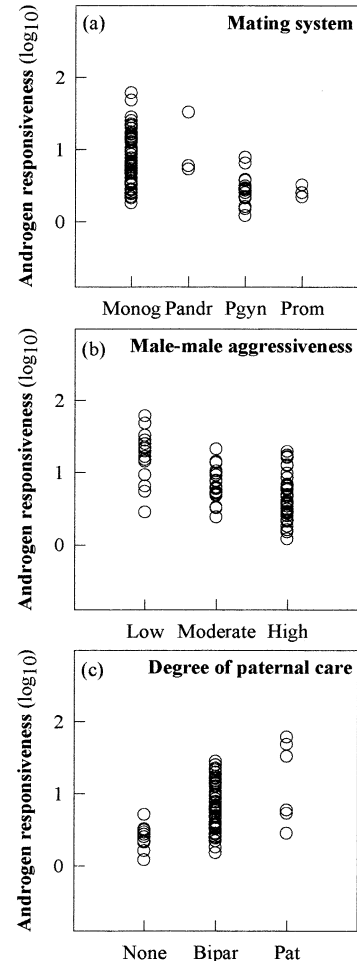

Fig. 2. Interspecific patterns of the AR (please refer to legend of Fig. 1 for details) by (a) mating system (Monog, monogamous; Pandr, polyandrous; Pgyn, polygynous; Prom, promiscuous), (b) male–male aggressiveness, and (c) by the degree of paternal care, (None, no paternal care; Bipar, biparental; Pat, paternal care.) The single effects of mating system (without phylogeny: $F_{3,80} = 9.91$; $P < 0.0001$; with phylogeny: $F_{3,80} = 7.37$; $P < 0.0005$), male–male aggressiveness (without phylogeny: $F_{2,81} = 21.34$; $P < 0.0001$; with phylogeny: $F_{2,81} = 16.34$; $P < 0.0001$) and the degree of paternal care (without phylogeny: $F_{2,81} = 15.08$; $P < 0.0001$; with phylogeny: $F_{2,81} = 15.08$; $P < 0.0001$) on AR remained significant after adding the phylogenetic information. However, in the case of paternal care, both analyses (without and with phylogeny) revealed that the major variation of AR was explained by the change from no paternal care to biparental care ($F_{1,82} = 25.95$; $P < 0.0001$), whereas the effect of the change from biparental to full paternal care did not remain significant ($F_{1,82} = 4.28$; $P > 0.05$).
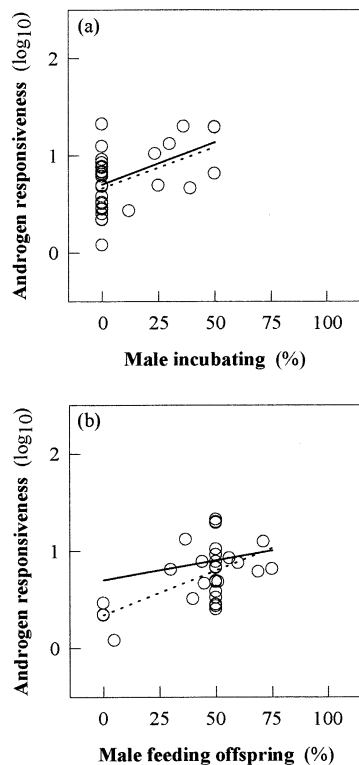
Fig. 3. Interspecific patterns of the AR (please refer to legend of Fig. 1 for details) among males of 32 passerine bird species by the contribution of the male parent during (a) incubation (without phylogeny: $F_{1,30} = 7.97$; $P < 0.01$; with phylogeny: $F_{1,30} = 7.16$; $P < 0.02$) and during (b) feeding the offspring (without phylogeny: $F_{1,30} = 16.29$; $P < 0.0005$: with phylogeny: $F_{1,30} = 1.46$; $P > 0.05$). Broken line represents the single regression on raw data; solid line represents the single regression including phylogenetic distances (GLS). Only the single effect of the males' contribution to incubation remained significant after control for phylogeny, whereas the effect of male chick feeding did not.

In line with this, there was a highly significant effect of all independent variables on the observed androgen responses, with a nonsignificant contribution of paternal care. Thus, the final model, which is also the best acording to Akaike's information criterium that takes the number of predictors into account (Efron and Tibshirani, 1993, Tabachnick and Fidell, 2001) included male–male aggressiveness and mating system only ($F_{7,76} = 5.69$; $P < 0.0001$) and suggested (as determined by the confidence limits) that only male–male aggressiveness and mating strategy were relevant for evolutionary changes of the AR.

Paternal investment among passerine species

Among the raw data of the subsample of passerine species ($N = 32$), the effects of incubation and feeding offspring were significant (Fig. 3a and b). Controlling for phylogeny within this group, however, only the single effect of the males' contribution to incubation was significant (Fig. 3a), while chick feeding was not (Fig. 3b). This demonstrated that the major variation of AR was due to the

evolutionary change from no male incubation to male incubation, rather than to the males' involvement during phases of feeding offspring between hatching and fledging.

Social environment

The observed androgen response rates were not different due to group living, either between species with dispersed breeding or those with breeding aggregations ($F_{1,71} = 0.008$; $P > 0.05$), or between species with solitarily living males or those with multimale groups ($F_{1,77} = 0.006$; $P > 0.05$). Males from species with either type of pairbond showed larger androgen response rates than males from species with no pairbond at all ($F_{1,77} = 7.91$; $P < 0.01$). Response rates of altricial and of precocial species were not significantly different ($F_{1,82} = 0.95$; $P > 0.05$).

Discussion

The challenge hypothesis proposes an interaction among mating strategy, the degree of paternal investment, territorial male–male aggressiveness, and the males' potential AR to social challenges at both the intraspecific and the interspecific levels (Wingfield et al., 1990, 2000). This study has focused on the predictions of the challenge hypothesis at the interspecific level.

The interspecific patterns described by Wingfield and co-workers remained relatively robust in our analysis with an enlarged sample size of avian species (Fig. 1). Because only a minor fraction of the sample originated from situational data, i.e., provided a classification of the male–male aggressiveness from simulated territorial intruder experiments (Wingfield, 1990; Table 2), we observed difficulties to reproduce the ratio d (i.e., male–male aggressiveness divided by the degree of paternal investment). In order to apply multivariate statistics, the effects of the different parameters (mating strategy, degree of paternal care, and male–male aggressiveness) were investigated separately.

Controlling for the phylogenetic relatedness (Felsenstein, 1985; Martins and Hansen, 1997; Pagel and Harvey, 1988) among all of the sampled species revealed that predominantly mating system and male–male aggressiveness were relevant for an evolutionary change of the AR. Also, repeating analyses with discrete variables for mating system (monogamous vs polygynous) confirmed these results. The effect of the degree of paternal care (categories as in Wingfield et al., 1990, 2000; Table 2) on the interspecific patterns of AR, however, was dramatically reduced. To check for the lack of paternal care effects we then repeated analyses of the effects of paternal investment using a quantified scale of male incubation and feeding offspring rates among the subsample of closely related passerine (altricial) species ($N = 32$). Results from this subsample revealed significant effects of the males' specific contribution during incubation on AR, but no effect of feeding offspring rates

(Fig. 3a and b). Although this indicated that paternal incubation seemed to interact with AR to some degree, the effect was observed only among a subsample of altricial species and may not have covered a representative fraction of the overall variability of mating systems (Table 2). Therefore, we consider the results originating from comparisons among the total sample (including precocial species) as generally more essential.

Although each of the approaches presented in this study has its own methodological flaw, they all result in the same robust pattern. It suggests that differences of AR have clearly evolved in response to variation in mating strategies and male–male aggression, whereas changes of AR in response to altered degrees of paternal investment appeared more complex. Among the subsample of the closely related passerines, only the change from no male incubation to male shares incubation contributed significantly to the evolution of different AR in response to territorial challenges. Therefore, the males' contribution during incubation may be regarded as the maximum paternal investment during all breeding phases, which was also reflected in the observed AR. This conclusion may sound contradictory to a large body of research that has shown a modulator role of testosterone in the trade-off between male–male aggressiveness and paternal care (specifically measuring feeding offspring rates) at the intraspecific level (Silverin, 1980; Wingfield, 1984b; Hegner and Wingfield, 1987; Beletsky et al., 1989; Dittami et al., 1991; Ketterson and Nolan, 1992; De Ridder et al., 2000), although several recent studies have suggested that low androgen levels are not a requirement for paternal behavior in all species (Hunt et al., 1999; Van Duyse et al., 2000, 2002; Lynn et al., 2002). However, the observed interactions between social environment and androgen responses do not have to act equally at both levels of analysis. Hence, the nonsignificant effect of the degree of paternal care in general on the flexibility of male AR using data controlled for phylogeny, stresses the importance of precisely adding the operational unit to the proposed social modulation of androgen levels in birds.

The trade-off between territorial male–male aggression and paternal care, of which flexible androgen patterns are the underlying mechanism, is defined for the fitness costs of mutually exclusive life-history decisions/states at an individual level (Stearns, 1989; Ketterson and Nolan, 1992). This trade-off was reflected in the presented interspecific comparisons as well. In the attempt to test for the basic trade-off assumptions of the challenge hypothesis, we observed a nonrandom pattern of male–male aggressiveness and paternal care (Table 3). Thus, it may be correct to consider the antagonistic nature of male–male aggressiveness and paternal care even at the interspecific level. Consequently, we may have observed the disappeared effect of paternal care on differences in AR independently of the seemingly remaining antagonistically interacting parameters.

The challenge hypothesis (Wingfield et al., 1990) also

Table 3
The trade-off between male–male aggressiveness and degree of paternal care among males from 84 bird species

Mm aggressiveness	Maternal	Biparental	Paternal
Low	0	13	4
Moderate	2	17	2
High	12	34	0

Note. Table shows the numbers of species per trait combination. Low male–male aggressiveness was observed exclusively among males with any paternal investment (biparental or paternal). In contrast, high male–male aggressiveness was observed predominantly among males from biparental and maternal species, but not among males from species with full paternal care.

offers a conceptual framework for attempts to explain the plasticity and the predictability of male androgen responses with parameters of the social environment, such as breeding dispersal, group living, social status (Beletsky et al., 1992; Wada et al., 1999), alternative life histories (Moore, 1991; Ketterson and Nolan, 1992; Oliveira et al., 2001b), and the predictability of the physical environment (Wingfield et al., 1993, 2000). Because species living at high group densities may be adapted to cope with higher frequencies of male–male interactions, the challenge hypothesis would predict higher basal androgen levels throughout the breeding phases and hence, smaller androgen increases in response to an additional social challenge as compared with solitary males (Beletsky et al., 1992; Pankhurst and Barnett, 1993; Wada et al., 1999; Wingfield et al., 1999a). Among the species included in the present study there was no effect either of the breeding dispersal (i.e., species with dispersed or aggregated nesting; Westneat and Sherman, 1997; Searcy et al., 1999) or of group living outside of the breeding season (i.e., species with solitary males or species with multimale groups; Moller and Birkhead, 1993) on the flexibility of AR to social challenges. This does not exclude the possibility that breeding dispersal and group composition still remain secondarily involved parameters by being related with male–male aggressiveness and mating strategy, which were shown to modulate the androgen response rates. Alternatively, this may add another example that explanations at an intraspecific level differ from those at the interspecific level.

In conclusion, our study pinpointed the importance of controlling for phylogenetic effects while examining relationships among phenotypic traits across species (Felsenstein, 1985; Harvey and Pagel, 1991; Martins and Hansen, 1997). Comparative studies allow to extend insights from studies at the population level and to generate predictions at the interspecific level (MacDougall-Shackleton and Ball, 1999; Dunn et al., 2001). The knowledge that changes of mating strategy and of territorial male–male aggressiveness rather than altered degrees of paternal care in general were related to the evolution of different androgen response rates in male birds, may contribute to the discussion of the applicability of the predictions of the challenge hypothesis throughout the vertebrates (vertebrates in general: Hirschen-

hauser and Oliveira, 2000; lizards: Thompson and Moore, 1992; but see Houck and Woodley, 1995; teleosts: Cardwell and Liley, 1991; Pankhurst and Barnett, 1993; Oliveira et al., 1996, 2001c, 2002; mammals: Cavigelli and Pereira, 2000; Nunes et al., 2000; but see Creel et al. 1993). Only the change to extremely high paternal investment (i.e., paternal incubation among the altricial passerines) contributed to the evolution of different androgen response rates. Finally, this study demonstrated that mating strategy and male–male aggressiveness are the primary functions of androgen-dependent male behavior, whereas paternal investment is not a function of but rather constrained by high androgen levels.

Acknowledgments

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